

# Prevalence and Phenotype Consequence of FRAXA and FRAXE Alleles in a Large, Ethnically Diverse, Special Education–Needs Population

Dana C. Crawford,<sup>1</sup> Kellen L. Meadows,<sup>1</sup> James L. Newman,<sup>1</sup> Lisa F. Taft,<sup>1</sup> Dorothy L. Pettay,<sup>1</sup> Laura B. Gold,<sup>1</sup> S. Jane Hersey,<sup>1</sup> Elizabeth F. Hinkle,<sup>1</sup> Mary L. Stanfield,<sup>1</sup> Patricia Holmgreen,<sup>2</sup> Marshalyn Yeargin-Allsopp,<sup>2</sup> Coleen Boyle,<sup>2</sup> and Stephanie L. Sherman<sup>1</sup>

<sup>1</sup>Department of Genetics, Emory University School of Medicine, and <sup>2</sup>Division of Child Development, Disability, and Health, Centers for Disease Control and Prevention, Atlanta

## Summary

We conducted a large population-based survey of fragile X (FRAXA) syndrome in ethnically diverse metropolitan Atlanta. The eligible study population consisted of public school children, aged 7–10 years, in special education–needs (SEN) classes. The purpose of the study was to estimate the prevalence among whites and, for the first time, African Americans, among a non–clinically referred population. At present, 5 males with FRAXA syndrome (4 whites and 1 African American), among 1,979 tested males, and no females, among 872 tested females, were identified. All males with FRAXA syndrome were mentally retarded and had been diagnosed previously. The prevalence for FRAXA syndrome was estimated to be 1/3,460 (confidence interval [CI] 1/7,143–1/1,742) for the general white male population and 1/4,048 (CI 1/16,260–1/1,244) for the general African American male population. We also compared the frequency of intermediate and premutation FRAXA alleles (41–199 repeats) and fragile XE syndrome alleles (31–199 repeats) in the SEN population with that in a control population, to determine if there was a possible phenotype consequence of such high-repeat alleles, as has been reported previously. No difference was observed between our case and control populations, and no difference was observed between populations when the probands were grouped by a rough estimate of IQ based on class placement. These results suggest that there is no phenotype consequence of larger alleles that would cause carriers to be placed in an SEN class.

## Introduction

Both fragile X (FRAXA) syndrome and fragile XE (FRAXE) syndrome are caused by the expansion of a trinucleotide repeat and are associated with a mental retardation phenotype. Although these syndromes may be linked by a common mechanism of expansion, they are very different in terms of prevalence, phenotype, and molecular basis of the syndrome.

### FRAXA Syndrome

The prevalence of FRAXA syndrome is ~1/4,000 males and 1/8,000 females (Turner et al. 1996). More than 95% of cases of FRAXA syndrome are caused by the hyperexpansion of a CGG trinucleotide repeat in the 5' UTR of the gene *FMR-1*, located at Xq27.3 (Fu et al. 1991; Verkerk et al. 1991). As a consequence of the hyperexpansion, the CpG island upstream of *FMR-1* becomes hypermethylated, and no message is produced (Pieretti et al. 1991; Sutcliffe et al. 1992; McConkie-Rosell et al. 1993). The absent gene product of *FMR-1*, called “FMRP,” normally is expressed as an RNA-binding protein found to be highly expressed in the brain and testis (Ashley et al. 1993).

In a normal population, the FRAXA CGG-repeat size is highly polymorphic, within the range of 6–54 repeats, and usually is inherited in a stable manner from parent to offspring (Fu et al. 1991). The CGG repeat is not a pure repeat and usually is interspersed with an AGG every 9–10 CGGs (Kunst and Warren 1994). The CGG-repeat sizes that are 60–200 repeats are termed “premutations,” because they tend to be inherited in an unstable manner and are a prelude to the full mutation. Alleles of 41–60 repeats (intermediate alleles) sometimes are considered to be in the “gray zone,” because they overlap the normal and premutation repeat ranges and may or may not be inherited unstably (Murray et al. 1996; Nolin et al. 1996; Zhong et al. 1996). Hyperexpansions of >200 repeats become hypermethylated and are termed “full mutations.” Almost all males with full mutations and 50% of females with full mutations

Received October 5, 1998; accepted for publication December 4, 1998; electronically published January 21, 1999.

Address for correspondence and reprints: Dr. Stephanie L. Sherman, Department of Genetics, Emory University, 1462 Clifton Road NE, Atlanta, GA 30322. E-mail: [ssherman@genetics.emory.edu](mailto:ssherman@genetics.emory.edu)

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0002-9297/99/6402-0019\$02.00

exhibit FRAXA syndrome (Wolff et al. 1988; Rousseau et al. 1994).

Males harboring the full mutation exhibit a wide range of phenotypes, including a broad forehead, an elongated face, large ears, and macro-orchidism. In terms of behavior, males often have autistic-like features, hyperactivity, tactile defensiveness, and a short attention span (Hagerman 1996). The degree to which a male with a full mutation is mentally impaired varies: mental retardation ranges from profound to mild, with most being moderately retarded (IQ 40-54) (Hagerman 1996).

Females with a full mutation often present with a milder phenotype, compared with males, presumably because of X inactivation (Wolff et al. 1988; Rousseau et al. 1994). Most often, affected females suffer from attention deficits, impulsivity, and learning disabilities, although a portion of affected females are overtly retarded (Hagerman 1992). Those with severe problems tend to have physical features similar to those of affected males (Hagerman et al. 1991).

Unlike individuals with full mutations, carriers with a premutation do not exhibit obvious clinical symptoms of FRAXA syndrome, presumably because their *FMR-1* gene is not transcriptionally silenced. However, there are data that suggest a phenotype consequence of the premutation alleles. To date, the phenotype most convincingly associated with premutation alleles is premature ovarian failure (POF), which is found to be significantly increased among female premutation carriers, compared with the relatives of both noncarriers and full-mutation carriers (Cronister et al. 1991; Schwartz et al. 1994; Conway et al. 1995; Partington et al. 1996; Conway et al. 1998; Murray et al. 1998; Allingham-Hawkins and International Collaborative POF in Fragile X Study Group, in press).

Milder features of the cognitive, behavioral, and phenotypic traits associated with the full-mutation allele have been reported to be associated with the premutation allele, although findings are inconclusive (Hull and Hagerman 1993; Mazzocco et al. 1993; Reiss et al. 1993; Loesch et al. 1994; Allingham-Hawkins et al. 1996; Franke et al. 1996; Riddle et al. 1998). Little is known about males with a premutation, but one study suggests that these males have large ears and deficits in nonverbal tasks, compared with non-fragile X carriers (Loesch et al. 1994). However, there is no molecular evidence for a phenotype associated with the premutation alleles, since it has been shown that *FMR-1* gene expression is comparable for normal and premutation alleles (Feng et al. 1995).

#### FRAXE Syndrome

FRAXE syndrome is caused by the expansion of a GCC repeat in the *FMR-2* gene (Knight et al. 1993).

The prevalence of FRAXE syndrome is estimated to be 1/50,000 males, which is ~14-fold less than the incidence of FRAXA syndrome (Brown 1996; Knight et al. 1996). The FRAXE locus, located at Xq27-28, is ~600 kb distal to *FMR-1* (Sutherland and Baker 1992; Flynn et al. 1993). Like *FMR-1*, *FMR-2* is associated with a nearby CpG island that is hypermethylated on hyperexpansion of the GCC repeat (Knight et al. 1993).

The FRAXE repeat is a pure repeat with no unique interspersed sequences (Zhong et al. 1996). Normal GCC alleles are 3-42 repeats. The border between the normal allele and the premutation allele is still poorly defined, because the FRAXE mutation is so rare.

Like those for FRAXA, full mutations for FRAXE were believed to be >200 repeats (Knight et al. 1993). However, recent studies have shown that the hyperexpansion and subsequent methylation threshold may be lower for the FRAXE locus (Hamel et al. 1994; Biancalana et al. 1996; Gecz et al. 1997). Furthermore, the full-mutation phenotype for FRAXE syndrome has been found to be much milder than the FRAXA phenotype. Among the few families that have been described, patients tended to have behavioral problems and speech delay (Dennis et al. 1992; Hamel et al. 1994; Mulley et al. 1995; Knight et al. 1996; Barnicoat et al. 1997). However, non-mentally impaired individuals who have the FRAXE full mutation and the consequent lack of *FMR-2* expression also have been reported in the literature (Gecz et al. 1997). No studies to date have been performed on FRAXE premutation carriers to assess the possible impact of this allele.

#### FRAXA and FRAXE: Phenotype Association with Normal Alleles

Like the premutation alleles, it is questionable whether the FRAXA and FRAXE intermediate alleles are associated with a phenotype. Murray et al. (1996) recently reported an excess of intermediate/premutation FRAXA (41-199 repeats) and FRAXE (31-199 repeats) alleles in a population of English boys in special education-needs (SEN) classes, when compared with maternal control X chromosomes. This excess suggests that there in fact may be a phenotype consequence of the intermediate and premutation alleles, at either locus.

Evidence against a phenotype consequence of large, unmethylated alleles is supported by at least two other studies. Mazzocco et al. (1997) and Mornet et al. (1998) both recently reported no excess of intermediate/premutation alleles in their respective populations. However, the former did not include controls, and the latter used a small sample size.

To examine the phenotype consequence of the FRAXA and FRAXE alleles, we surveyed a large, ethnically diverse population of school children in SEN classes in

metropolitan Atlanta. The purpose of this study is several-fold: (1) to determine the frequency of the full-mutation allele in both whites and African Americans in SEN classes, as well as in the general population; (2) to determine the phenotype of the full-mutation carrier in this non-clinically referred population; and (3) to compare the frequency of the premutation and intermediate alleles in an SEN population with that in a control population, to determine if there is a possible phenotype consequence related to cognitive ability and/or behavior. We found that the prevalences of FRAXA syndrome, premutation carriers, and the frequency of intermediate alleles were similar to those reported previously. Furthermore, we found that the prevalence of FRAXA syndrome among African American males appears to be similar to that among white males, in metropolitan Atlanta, which is  $\sim 1/4,000$ . All identified males had the classic phenotype of FRAXA syndrome. Last, we found no evidence of an increased frequency of intermediate/premutation alleles among FRAXA or FRAXE probands, when compared with a control population of maternal untransmitted alleles.

## Subjects and Methods

### Study Population

The target population comprised public school children, aged 7–10 years, in SEN classes in four of the five districts of metropolitan Atlanta. The recruiting process for the target population has been described elsewhere (Meadows et al. 1996). A buccal brush or mouth-wash sample was obtained from each participating child, and a separate invitation packet requesting buccal brush samples was sent to the mother and father of each child.

### Laboratory Methods

FRAXA and FRAXE allele sizes were determined by a fluorescent method described elsewhere (Meadows et al. 1996). Male samples that failed to amplify and female samples that revealed a single band for FRAXA, by means of this method, were subjected to a second PCR protocol, described elsewhere (Brown et al. 1993). We were unable to pursue those samples that did not yield results when the method described for FRAXE was used. If the second PCR procedure did not yield conclusive results for the sample in question, the individual was approached for a blood sample, to examine the FRAXA locus by use of a Southern blot protocol (Meadows et al. 1996).

If an individual with inconclusive results was unable to submit a blood sample, the DNA from the buccal or mouth-wash sample was used to test for the expanded FRAXA alleles, by use of Expand Long Template Polymerase (Boehringer Mannheim). The DNA for each sam-

ple was diluted to 1 ng/ $\mu$ l, and 1  $\mu$ l of the diluted sample was used for each PCR reaction. Amplification of genomic DNA was performed with 0.7 mM each of primers C and F, in a 15- $\mu$ l cocktail mix of 1  $\times$  assay buffer containing 1.75 mM  $MgCl_2$  (Boehringer Mannheim); 10% dimethyl sulfoxide; 500 mM dATP, dCTP, dTTP, and 7-deaza dGTP; 0.25 mM  $MgCl_2$ ; and 1 U Expand Long Template Polymerase (Boehringer Mannheim). The reactions were heated to 95°C for 10 min, followed by five cycles of denaturation at 95°C for 2.5 min, annealing at 65°C for 1 min, and elongation at 72°C for 2.5 min. The samples were further subjected to 25 cycles of denaturation at 95°C for 1.5 min, annealing at 55°C for 1 min, and elongation at 72°C for 2.5 min, followed by a final elongation step at 72°C for 5 min. Five microliters of loading dye was added to the PCR reaction and was denatured at 95°C for 5 min before being loaded onto a 6% acrylamide gel (Gibco-BRL). The gel (17  $\times$  17 cm) was run for  $\sim 1$  h 15 min. The DNA was transferred to a positively charged membrane (Boehringer Mannheim) and was prehybridized and hybridized in accordance with the manufacturer's instructions. The probe (CGC)<sub>7</sub> was 3' end labeled in accordance with the manufacturer's instructions. After the application of CDP-STAR (Boehringer Mannheim), the DNA was visualized by use of autoradiography, typically after a 3–15-min exposure. For each gel, a set of controls, including a blank control (double-deionized water), and samples from normal, premutation, and full-mutation individuals of each sex were run. Consistent with our results, it has been shown previously that the Expand Long PCR procedure can be used to amplify premutation and full-mutation alleles (Hecimovic et al. 1997).

### Statistical Methods

A  $\chi^2$  test of independence or a Fisher's exact test using the software StatXact (Cytel) was performed to compare genotypes or allele distributions. The difference between the observed and expected heterozygosities was tested as described elsewhere (Meadows et al. 1996).

Power calculations were performed by use of the following equation:

$$Z_{(.05)} = 2[|\arcsine(p_1)^{1/2} - \arcsine(p_2)^{1/2}|] \\ \times [(N_1 N_2)/(N_1 + N_2)]^{1/2} - 1.96,$$

where  $p_1$  and  $p_2$  are the frequencies of intermediate/premutation alleles in case and control populations, respectively,  $N_1$  is the sample size of the case population, and  $N_2$  is the sample size of the control population (Motulsky 1995). For purposes of comparison between the present study and that of Murray et al. (1996), we set  $p_1 = .0355$  and  $p_2 = .019$  for the FRAXA locus and  $p_1 = .011$  and  $p_2 = .0028$  for the FRAXE locus. In cal-

culating the 95% confidence intervals (CIs), we used the equations recommended by Fleiss (1981), because the frequencies of the full-mutation, premutation, and intermediate alleles were near 0.

## Results

### Study Population

Eligible subjects were children, aged 7–10 years, who attended SEN classes (i.e., had an active individual educational program) in the public school system of Atlanta. We excluded children in resource speech, as well as those in gifted classes. Both males and females and all ethnic groups were included in this study. Subjects were not excluded on the basis of known etiology. The participation rate was 46% (table 1). The study population was comparable to the target population, with regard to sex, ethnicity, and primary educational need (table 2 and data not shown). The participation rate of mothers and fathers in our study was 35% and 27%, respectively (table 1). We obtained samples from 2,873 of the 2,956 children who had parental consent. The remaining 83 cases moved out of the school system before a sample was obtained and could not be tracked, owing to lack of a forwarding address. Of the DNA samples received, 22 (0.77%) failed to give FRAXA results, and 221 (7.69%) failed to give FRAXE results. Unlike for the FRAXA locus, we did not attempt to identify further the carrier status of females who had a single band or individuals who repeatedly failed to amplify for the FRAXE locus. Thus, we were unable to identify any FRAXE full-mutation individuals and possibly some FRAXE premutations in samples that did not amplify by use of the primary PCR method (see Subjects and Methods). Since we were able to follow up all FRAXA failed samples, using a more sensitive assay, we found that the failure to amplify at the FRAXA locus probably was not due to an expanded allele in these samples. Rather, the DNA most likely was of poor quality, since we could not amplify the surrounding markers in these samples. In testing for the FRAXA and FRAXE loci, we identified several sex-chromosome abnormalities in our SEN population (table 3). We identified one 47,XXX female (0.03%) and seven 47,XXY males (0.24%). We also had two Turner syndrome females (0.07%) who had been diagnosed previously, as indicated on their consent forms. These findings are in agreement with those of other studies (Dawson et al. 1995; Holden et al. 1995).

### FRAXA: Prevalence

We identified five FRAXA full-mutation males and no females. One of the five full-mutation males was African American, and the others were white. All these full-mu-

**Table 1**

**Sample Size and Participation Rates, by District**

| DISTRICT | SAMPLE SIZE (PARTICIPATION RATE) |             |           |
|----------|----------------------------------|-------------|-----------|
|          | Probands                         | Mothers     | Fathers   |
| 1        | 902 (.39)                        | 360 (.40)   | 281 (.31) |
| 2        | 551 (.51)                        | 184 (.34)   | 126 (.23) |
| 3        | 469 (.43)                        | 127 (.26)   | 80 (.17)  |
| 4        | 1,034 (.52)                      | 369 (.36)   | 296 (.29) |
| Total    | 2,956 (.46)                      | 1,040 (.35) | 783 (.27) |

NOTE.—The participation rates of the parents include only those parents whose child provided a sample.

tation individuals had been diagnosed previously; thus, we did not identify any new cases of FRAXA syndrome in this survey. All had a typical fragile X phenotype for males, and all were in SEN classes, because of mental retardation. For white and African American males in an SEN population, we calculated the prevalence of FRAXA syndrome to be 1/362 (CI 1/1,129–1/132) and 1/422 (CI 1/8,066–1/66), respectively (table 4). Although the confidence limits are wide, there was no difference in rates between white and African American males. To calculate the prevalence in the general population, we assumed that all individuals with FRAXA syndrome would be found in SEN classes; that is, we did not expect individuals with FRAXA syndrome to be attending non-SEN classes. Furthermore, we assumed that the rate of individuals with the syndrome was the same in the participating SEN population as in the non-participating SEN population. On the basis of these assumptions, we estimated the overall prevalence among males to be 1/3,968 (CI 1/7,353–1/2,188).

We identified only two FRAXA premutations, and both were in white females. Thus, the prevalence of FRAXA premutation carriers among white females was estimated to be 1/317 (CI 1/832–1/79). Although we did not identify any new cases of FRAXA syndrome among probands, we did identify a new case of FRAXA syndrome through one of the newly identified female FRAXA premutation carriers. We did not identify any male FRAXA premutation carriers among whites or African Americans in our SEN population (table 4).

Because the intermediate alleles (41–60 repeats) are thought to be precursors of premutation alleles, we examined the prevalence of carriers among both whites and African Americans (table 4). Although not statistically different, the prevalence among male and female carriers was lower for African Americans, compared with whites. Furthermore, among African Americans the ratio of female to male carriers of intermediate alleles was 0.72:1, whereas among whites the ratio (1.46:1; table 4) was closer to the 2:1 ratio expected for an X-linked neutral allele.

**Table 2****Description of Study Population: Frequencies for Sex, Ethnicity, and Primary Educational Need, by District**

| DISTRICT | FREQUENCY |     |              |     |     |                               |                   |                   |                       |                    |
|----------|-----------|-----|--------------|-----|-----|-------------------------------|-------------------|-------------------|-----------------------|--------------------|
|          | Sex       |     | Ethnic Group |     |     | Primary Educational Need      |                   |                   |                       |                    |
|          | M         | F   | W            | AA  | O   | Emotional Behavioral Disorder | Learning Disabled | Mentally Retarded | Other Health Impaired | Other <sup>a</sup> |
| 1        | .68       | .32 | .80          | .16 | .04 | .18                           | .53               | .11               | .14                   | .04                |
| 2        | .67       | .33 | .58          | .36 | .07 | .15                           | .60               | .14               | .05                   | .06                |
| 3        | .69       | .31 | .57          | .38 | .05 | .21                           | .43               | .29               | .00                   | .07                |
| 4        | .72       | .28 | .82          | .10 | .07 | .25                           | .43               | .13               | .12                   | .07                |
| Total    | .70       | .30 | .73          | .21 | .06 | .20                           | .49               | .15               | .09                   | .06                |

NOTE.—M = male, F = female, W = white, AA = African American, and O = Other.

<sup>a</sup> Autistic, orthopedically impaired, deaf and/or blind, visually or hearing impaired, traumatic brain injury, or self-contained speech.*FRAXE: Prevalence*

As discussed in Subjects and Methods, we were not able to detect FRAXE full-mutation carriers by using our assay. We identified only one male premutation carrier of 63 repeats, who was African American, and no female carriers. Because we did not follow up the FRAXE PCR failures, this number may represent an underestimation (table 5). We also examined the frequency of the intermediate alleles (31–60 repeats) and found that the ratio of female to male carriers was 1:1.29 for whites and 1.5:1 for African Americans (table 5). These carrier ratios should be interpreted with caution, because the number of FRAXE intermediate alleles was quite small for both ethnic groups (whites,  $n = 16$ ; African Americans,  $n = 5$ ).

*FRAXA: Allele Distribution and Heterozygosity*

All probands were used to generate allele distributions for the FRAXA locus ( $n = 3,724$  alleles). The overall distribution for the FRAXA locus was similar to that reported elsewhere (Meadows et al. 1996). The allele distribution for whites was then compared with that for African Americans. Overall, the FRAXA distribution for whites ranged from 11 to >200 repeats and had 52 distinct alleles, whereas the distribution for African Americans had an identical range but only 34 distinct alleles (table 6). Interestingly, the difference in the number of distinct alleles between the two ethnic groups was driven by the diversity of the intermediate alleles in whites: there were 20 distinct intermediate alleles in whites and 6 distinct intermediate alleles in African Americans. To better understand the difference between the two distributions and to test for significance, the allele sizes were collapsed into three categories (11–26 repeats, 27–34 repeats, and >34 repeats) based on the antimode of the overall distribution for the FRAXA locus. A significant difference between whites and African Americans was observed for

the FRAXA alleles ( $\chi^2 = 61.7$ ,  $P < .001$ ), and this difference was primarily due to two observations: there was a lower frequency of smaller alleles, such as alleles of 20 and 23 repeats, and a lower frequency of alleles >40 repeats in the African American population, compared with whites. In fact, with respect to the allele frequency of the intermediate alleles (41–60 repeats), only 2.2% of the African American alleles fell within this category, whereas 4.0% of the white alleles fell within the same category, as is reflected by the carrier frequencies for these alleles, shown in table 4.

Hardy-Weinberg equilibrium for the FRAXA locus was then examined in each population. To do this, the level of heterozygosity for each population was examined and compared with that predicted from the allele distribution for males (see Statistical Methods). At the FRAXA locus, African Americans showed reduced levels of heterozygosity, whereas whites fit the expected level (table 6).

*FRAXE: Allele Distribution and Heterozygosity*

As we did for FRAXA, we collapsed the FRAXE distribution ( $n = 3,431$  alleles) into three categories (3–11 repeats, 12–20 repeats, and >20 repeats) based on the antimode of the overall distribution, to test for a significant difference between the two populations. A difference between whites and African Americans was observed at the FRAXE locus ( $\chi^2 = 24.7$ ,  $P < .001$ ), and this difference was primarily due to the higher frequency of smaller alleles in the African American population, compared with whites. Overall, the FRAXE distribution for whites was 7–54 repeats and had 34 distinct alleles, whereas the distribution for African Americans had a similar range and only 27 distinct alleles (table 6). The diversity of alleles in the distribution for whites, compared with that in the distribution for African Ameri-

**Table 3****Study Populations by Sex and Ethnicity, for FRAXA**

| POPULATION           | NO. OF MALES |        |        |       | NO. OF FEMALES |        |        |       |
|----------------------|--------------|--------|--------|-------|----------------|--------|--------|-------|
|                      | Total        | W      | AA     | O     | Total          | W      | AA     | O     |
| General <sup>a</sup> | 46,523       | 30,653 | 11,667 | 4,203 | 44,444         | 29,055 | 11,432 | 3,957 |
| Target <sup>b</sup>  | 4,648        | 3,207  | 1,214  | 227   | 1,816          | 1,260  | 465    | 91    |
| Study:               |              |        |        |       |                |        |        |       |
| Full mutation        | 5            | 4      | 1      | 0     | 0              | 0      | 0      | 0     |
| Premutation          | 0            | 0      | 0      | 0     | 2              | 2      | 0      | 0     |
| Intermediate         | 78           | 64     | 13     | 1     | 48             | 41     | 4      | 3     |
| Common               | 1,889        | 1,373  | 407    | 109   | 819            | 590    | 175    | 54    |
| XXY                  | 7            | 6      | 1      | 0     | 0              | 0      | 0      | 0     |
| XXX                  | 0            | 0      | 0      | 0     | 1              | 1      | 0      | 0     |
| XO                   | 0            | 0      | 0      | 0     | 2              | 1      | 1      | 0     |
| Total                | 1,979        | 1,447  | 422    | 110   | 872            | 635    | 180    | 57    |

NOTE.—W = white, AA = African American, and O = Other.

<sup>a</sup> Children, aged 7–10 years, in the public school system during the time of the survey of each district.

<sup>b</sup> Children, aged 7–10 years, in SEN classes.

cans, was driven, like at the FRAXA locus, by the diversity of the intermediate alleles within that population.

The observed heterozygosity at the FRAXE locus was compared with that predicted by the allele distribution for males. Unlike for the FRAXA locus, reduced levels of heterozygosity were observed in both populations (table 6).

#### *Testing for Phenotype Consequence of Normal FRAXA and FRAXE Alleles*

Murray et al. (1996) recently demonstrated that there was an excess of intermediate/premutation FRAXA (41–199 repeats) and FRAXE (31–199 repeats) alleles in their SEN population of boys from Wessex, England. In view of these results, it was suggested that there could be a phenotype associated with these large, unmethylated alleles. To test this possibility in our SEN population, the frequency of intermediate/premutation FRAXA and FRAXE alleles among the probands was compared with that among controls, by use of a “matched” and “unmatched” approach. For the matched approach, the transmission of alleles from mother to proband was examined. The allele transmitted to the proband was considered the “case” allele, and the allele not transmitted to the proband was considered the “control” allele. For probands identified in this study as siblings, each transmission was considered an independent event. For this analysis, the population was not separated by ethnicity, because the control alleles served as ethnic matches to the case alleles. The case and control alleles were categorized according to repeat sizes similar to those used by Murray et al. (1996). In this SEN population, intermediate/premutation alleles were slightly more frequent in the case population for the FRAXA locus; however, this difference did not reach statistical

significance ( $P = .6981$ ; table 7). There was no difference between the case and control populations for the FRAXE locus ( $P = 1.000$ ; table 7). For the unmatched approach, all proband alleles, not just those from maternal samples, were considered the case alleles. The population of maternal untransmitted alleles, as well as of paternal untransmitted alleles, was used as the control population. For this analysis, both cases and controls were separated by ethnicity. We found no difference between the unmatched case and control populations, for either whites or African Americans, at the FRAXA locus ( $P = .8447$  and  $P = .7600$ , respectively; table 7) or at the FRAXE locus ( $P = 1.000$  and  $P = .6161$ , respectively; table 7).

Because these data contradict those of Murray et al. (1996), we further investigated our population to uncover any possible heterogeneity by school district or by IQ level. First, we analyzed our matched case and control populations by district. There was no significant statistical evidence of heterogeneity ( $P = .06$ ; table 8); however, the frequencies did fluctuate from district to district. Second, we analyzed our matched case and control populations according to a rough estimate of IQ. Our study encompassed many learning-impaired phenotypes, among which mental retardation accounted for only 15% of the study population (table 3). Intermediate/premutation alleles may be associated with a more severe phenotype, such as  $IQ < 70$ . Because we did not have access to school records, we based IQ on class placement. For some individuals we were unable to ascertain class placement. Those who were placed in classes because of mental retardation, severe behavioral disorders, and autism were classified as  $IQ < 70$ , and the remainder were classified as  $IQ \geq 70$ . The frequency of intermediate/premutation alleles was essentially the same for

**Table 4****Prevalence of FRAXA Full-Mutation, Premutation, and Intermediate Alleles**

| POPULATION <sup>a</sup>    | PREVALENCE (95% CI) AMONG MALES |                              |                               | PREVALENCE (95% CI) AMONG FEMALES |                         |                      |
|----------------------------|---------------------------------|------------------------------|-------------------------------|-----------------------------------|-------------------------|----------------------|
|                            | Total                           | W                            | AA                            | Total                             | W                       | AA                   |
| Full mutation:             |                                 |                              |                               |                                   |                         |                      |
| Target                     | 1/396<br>(1/1,074–1/160)        | 1/362<br>(1/1,129–1/132)     | 1/422<br>(1/8,066–1/66)       | 0/872 <sup>c</sup>                | 0/635 <sup>c</sup>      | 0/180 <sup>c</sup>   |
| General                    | 1/3,968<br>(1/9,353–1/2,188)    | 1/3,460<br>(1/7,143–1/1,742) | 1/4,048<br>(1/16,260–1/1,244) | ...                               | ...                     | ...                  |
| Premutation: <sup>b</sup>  |                                 |                              |                               |                                   |                         |                      |
| Target/general             | 0/1,979                         | 0/1,447                      | 0/422                         | 1/436<br>(1/2,519–1/109)          | 1/317<br>(1/1,832–1/79) | 0/180                |
| Intermediate: <sup>b</sup> |                                 |                              |                               |                                   |                         |                      |
| Target/general             | 1/25<br>(1/32–1/20)             | 1/23<br>(1/29–1/18)          | 1/32<br>(1/58–1/19)           | 1/18<br>(1/24–1/14)               | 1/15<br>(1/21–1/11)     | 1/45<br>(1/140–1/17) |

NOTE.—W = white, AA = African American, and O = Other.

<sup>a</sup> Target = children, aged 7–10 years, in SEN classes. General = children, aged 7–10 years, in the public school system during the time of the survey of each district.

<sup>b</sup> Prevalence in the general population was assumed to be that found in the target population, since no differences between cases and controls were found (see text).

<sup>c</sup> No individuals were observed; thus, prevalences could not be estimated.

both groups. We also examined this possible association by district, since each district has different criteria for placement. Again, we found no evidence of heterogeneity, although estimates did fluctuate (data not shown).

## Discussion

### Prevalence

The prevalence 1/3,460 (CI 1/7,143–1/1,742) estimated here for FRAXA syndrome in a white male population is in agreement with that determined in previous studies (Murray et al. 1996; Turner et al. 1996; de Vries et al. 1997; Morton et al. 1997; Syrrou et al. 1998). On the basis of this estimate, we project the prevalence among females to be ~1/9,000, assuming that ~50% of female full-mutation carriers exhibit the syndrome (Rousseau et al. 1994) and that the frequency of male and female full-mutation carriers is equal (Sherman 1995a). Consistent with this expectation and our limited sample size, we did not identify any females with a full-mutation allele. Interestingly, no new FRAXA syndrome cases were discovered among the probands, although one undiagnosed male was ascertained through a female proband with a premutation and was identified by use of the Expand Long PCR procedure. Because we were specifically targeting children, aged 7–10 years, in SEN classes, the lack of new cases indicates that the vast majority of cases are being diagnosed before age 7 years.

As mentioned previously, to calculate the prevalence of FRAXA syndrome in the general population, we assumed that the numbers of FRAXA syndrome cases in our participating and nonparticipating populations were equal. Using the school record-surveillance system of

developmental disabilities established by the Centers for Disease Control and Prevention (CDC), we were able to identify the number of males and females with FRAXA syndrome who met the proper criteria at the time of ascertainment but who did not participate in our study. Two males (districts 1 and 4), one female (district 1) with FRAXA syndrome, and one female (district 1) with possible FRAXA syndrome did not participate in our survey. Thus, our assumption seemed to be confirmed. However, there is still a possibility that mothers who express the FRAXA syndrome were less willing both to participate and to pursue a diagnosis for their child; thus, these cases would be missed by both our screening study and the CDC surveillance. If true, our prevalence estimate is an underestimate of the true rate.

Anecdotally, there is a lack of African Americans with FRAXA syndrome reported in the literature (Howard-Peebles and Stoddard 1980; Venter et al. 1981; Venter and Op't Hof 1982). This could be due to either a true lower prevalence, resulting from the mutation history of the population, or an underascertainment of cases. We identified one African American male with a FRAXA full mutation who had been diagnosed previously. The resulting prevalence for this population was similar to that for whites, that is, 1/4,048 (CI 1/16,260–1/1,244). To our knowledge, this is the only reported estimate for African Americans that is based on a large, non-clinically referred population survey using a molecular diagnosis for FRAXA syndrome. A recent study in South Africa gave similar results: the prevalence of FRAXA syndrome among institutionalized South African blacks was similar to that reported in the literature for institutionalized white populations (Goldman et al. 1997).

**Table 5****Study Populations by Sex and Ethnicity, for FRAXE**

| POPULATION           | NO. OF MALES |        |        |       | NO. OF FEMALES |        |        |       |
|----------------------|--------------|--------|--------|-------|----------------|--------|--------|-------|
|                      | Total        | W      | AA     | O     | Total          | W      | AA     | O     |
| General <sup>a</sup> | 46,523       | 30,653 | 11,667 | 4,203 | 44,444         | 29,055 | 11,432 | 3,957 |
| Target <sup>b</sup>  | 4,648        | 3,207  | 1,214  | 227   | 1,816          | 1,260  | 465    | 91    |
| Study:               |              |        |        |       |                |        |        |       |
| Full mutation        | 0            | 0      | 0      | 0     | 0              | 0      | 0      | 0     |
| Premutation          | 1            | 0      | 1      | 0     | 0              | 0      | 0      | 0     |
| Intermediate         | 11           | 9      | 2      | 0     | 11             | 7      | 3      | 1     |
| Common               | 1,847        | 1,350  | 395    | 102   | 773            | 565    | 161    | 47    |
| XXY                  | 7            | 6      | 1      | 0     | 0              | 0      | 0      | 0     |
| XXX                  | 0            | 0      | 0      | 0     | 0              | 0      | 0      | 0     |
| XO                   | 0            | 0      | 0      | 0     | 2              | 1      | 1      | 0     |
| Total                | 1,866        | 1,365  | 399    | 102   | 786            | 573    | 165    | 48    |

NOTE.—W = white, AA = African American, and O = Other.

<sup>a</sup> Children, aged 7–10 years, in the public school system during the time of the survey of each district.

<sup>b</sup> Children, aged 7–10 years, in SEN classes.

Furthermore, Pulliam et al. (1988) showed that the percentage of institutionalized African American males with FRAXA syndrome who were diagnosed cytogenetically agreed with the percentage of African American males in the general population of South Carolina. Finally, a recent study by Elbaz et al. (1998) showed that the prevalence of FRAXA syndrome among a mostly Afro-Caribbean male population was similar to that among whites. These findings, as well as the prevalence reported here, suggest that the prevalence of FRAXA syndrome among white and African American populations does not differ.

With respect to premutation carriers, Rousseau et al. (1995) screened 10,624 unselected French Canadian women and estimated the prevalence of premutation carriers (55–199 repeats) to be 1/259 women (95% CI 1/373–1/198). If only those with  $\geq 61$  repeats are defined as premutation carriers, the estimate is 1/379. When these results were reported, two explanations were offered to explain the high prevalence of carriers in the Quebec population: (1) a founder effect in the French Canadian population and (2) a true high prevalence of carriers of the FRAXA premutation allele (Sherman 1995b). On the basis of our prevalence estimate of 1/317 (CI 1/1,832–1/79) in this survey of an admixed, presumably northern European white population, the latter explanation is the more likely of the two possibilities.

We did not identify any male FRAXA premutation carriers in our proband population. However, we did identify two male premutation carriers among our control population of 433 fathers ascertained through sons. Because our analyses showed that the allele distribution in our proband population did not differ from that in our control population, we combined these populations,

for a prevalence of white male premutation carriers of 1/923 (CI 1/5,319–1/229). Murray et al. (1996) also reported 1 male premutation carrier among 1,013 males in Wessex, England. We did not identify any FRAXA premutation carriers among our African American population.

The higher frequency of premutation alleles among females, versus males, in this population, as well as among the French Canadian population (Rousseau et al. 1995), was predicted from models that assume that expansion of the CGG repeat occurs meiotically, not post-zygotically; that is, for models that assume prezygotic expansion and subsequent selection against sperm with full mutations, the ratio of female to male premutation carriers is predicted to be  $\sim 2.6$  (Winter 1987; Sved and Laird 1990; Morton and Macpherson 1992; Kolehmainen 1994; Morris et al. 1995a, 1995b). In contrast, the model that assumes postzygotic expansion restricted to maternal X chromosomes predicts the female to male ratio to be  $\sim 1.2$  (Ashley and Sherman 1995). The prevalence estimates from our survey reflect a 2.9 ratio, which provides indirect evidence for the prezygotic expansion model.

Because the intermediate alleles may represent the alleles predisposed to expand to the premutation state, we calculated the prevalence of these alleles in both populations. Interestingly, the African American population had a lower prevalence of these alleles (2.2%), compared with whites (4.0%). It has been proposed that, for other trinucleotide-repeat diseases, such as myotonic dystrophy (DM), the smaller frequency of intermediate alleles leads to a lower prevalence of the disease in that particular ethnic population (Ashizawa and Epstein 1991; Goldman et al. 1994; Watkins et al. 1995). Although our African American population had a lower preva-



**Table 6**  
**Description of FRAXA and FRAXE Distributions among Whites and African Americans**

|                   | NO. OF ALLELES |        |       | HETEROZYGOSITY |                          |
|-------------------|----------------|--------|-------|----------------|--------------------------|
|                   | Mean           | Range  | Total | Expected       | Observed                 |
| FRAXA:            |                |        |       |                |                          |
| Whites            | 29.57          | 11–200 | 52    | .813 ± .010    | .823 ± .011              |
| African Americans | 30.11          | 11–200 | 34    | .812 ± .019    | .760 ± .023 <sup>a</sup> |
| FRAXE:            |                |        |       |                |                          |
| Whites            | 17.59          | 7–54   | 34    | .858 ± .009    | .786 ± .012 <sup>a</sup> |
| African Americans | 16.75          | 7–63   | 27    | .842 ± .018    | .767 ± .023 <sup>a</sup> |

<sup>a</sup> *P* < .05 (two-sided *z*-test).

lence of intermediate alleles, compared with whites, this population did not have a lower prevalence of disease alleles. This contrast to other repeat diseases needs to be confirmed, since the confidence limit was large in our estimate of the prevalence of FRAXA syndrome in the African American population. Although there are fewer intermediate alleles in the African American population, the proportion of alleles that contain a CGG/AGG-repeat structure susceptible to expansion may be equal, compared with that in the white population. Sequence data on African American intermediate alleles are necessary to confirm or deny this hypothesis.

*Possible Phenotype Consequence of Normal Alleles*

We found no evidence for an excess of intermediate/premutation alleles at the FRAXA and FRAXE loci in an SEN population, in contrast to the findings of Murray et al. (1996), the only other large study of an SEN population with controls. It is important to try to understand the differences between the two studies, since one explanation is a phenotype consequence of high-repeat alleles. If true, the result would have important implications in terms of both the impact on cognition and/or behavior (Teague et al. 1998) and the biological role of the CGG-repeat region in these genes. Our investigation was comparable both in the definition of cases (individ-

uals in SEN classes) and controls (maternal X chromosomes) and in the definition of intermediate/premutation alleles (>40 repeats for FRAXA and >30 repeats for FRAXE). However, the target populations clearly differed. In our study, both boys and girls, aged 7–10 years, were included, whereas Murray et al. included boys aged 5–18 years. Also, the proportion of individuals with mental retardation was higher in the study by Murray et al. (~33%; P. A. Jacobs, personal communication), compared with that in our population (~15%), although both estimates were crude. The proportions of individuals and the criteria of other types of educational needs also probably differed between the Atlanta and Wessex populations. Last, our population consisted of many ethnic groups, the two most prominent being whites and African Americans, whereas the population studied by Murray et al. consisted almost exclusively of whites.

If there is a phenotype consequence of intermediate/premutation alleles, any or all of these differences between populations could result in differences in the power to identify an effect. For example, the increased age structure of the population studied by Murray et al. (1996) could increase the power to detect a gene that plays a role in the cognitive ability and/or behavioral profile of an individual. Behavioral genetic studies of

**Table 7**  
**Frequency of Intermediate/Premutation Alleles among Cases and Controls, in a Matched and Unmatched Design**

| STUDY DESIGN<br>AND ALLELE TYPE | ALLELE FREQUENCY   |                   |                    |                   |
|---------------------------------|--------------------|-------------------|--------------------|-------------------|
|                                 | FRAXA, ≥41 Repeats |                   | FRAXE, ≥31 Repeats |                   |
| Matched:                        |                    |                   |                    |                   |
| Transmitted                     | 33/844 (.04)       |                   | 4/657 (.01)        |                   |
| Untransmitted                   | 29/844 (.03)       |                   | 4/657 (.01)        |                   |
|                                 | Whites             | African Americans | Whites             | African Americans |
| Unmatched:                      |                    |                   |                    |                   |
| Proband                         | 110/2,718 (.04)    | 17/782 (.02)      | 18/2,502 (.01)     | 6/725 (.01)       |
| Control <sup>a</sup>            | 37/891 (.04)       | 2/127 (.02)       | 6/887 (.01)        | 0/102             |

<sup>a</sup> Maternal untransmitted alleles and paternal untransmitted alleles, ethnically matched.

many traits show that heritability estimates are higher for adults, compared with those for children (McCartney et al. 1990). With respect to the proportion of individuals with mental retardation, neither our study (table 8) nor that of Murray et al. (P. A. Jacobs, personal communication) showed an association with the crude categorization of mental retardation. However, neither study had the ability to examine an association with severity or types of behavioral problems. Last, the frequency of genes that may modify the action of the FRAXA allele or that may be modified by the increased repeat number might differ between studies, because of the ethnic variation.

Another explanation for the difference in the ability to detect an excess of intermediate/premutation alleles simply could be related to the sample size. We examined the frequency of the tail of the distribution, a frequency that is <5% of the population; thus, large numbers are needed to detect significant differences. In this respect, the African American component of our study population could have had a major impact on the detection of an excess of intermediate/premutation alleles, since this population, in general, has been shown to have a lower frequency of these particular alleles. Our data did show the same trend of an excess of intermediate/premutation alleles at the FRAXA locus, but these data failed to reach significance. Because we failed to reject the null hypothesis, we calculated the power of this study to detect the difference observed by Murray et al. (1996) (see Statistical Methods). For the unmatched case/control FRAXA analysis of whites, the present study had a 75% probability to detect a difference of 1.65% between case and control populations, when the control frequency was assumed to be 1.9%. For FRAXE, the present study had a 72% probability to detect a difference of 0.82%, when the control frequency was assumed to be 0.28%.

The alternative possibility is that there is no phenotype consequence of intermediate/premutation alleles and that the results of Murray et al. (1996) simply are due

to statistical fluctuation. Evidence that supports the idea of statistical fluctuation is the fact that the frequencies of intermediate/premutation alleles among control maternal X chromosomes in the present study and in the study by Murray et al. are different. We found that 3% of control alleles are in the intermediate/premutation range, which is similar to that estimated in other studies of general populations (Dawson et al. 1995; Holden et al. 1995; Spence et al. 1996; Mornet et al. 1998; Haddad et al., in press). However, in the study by Murray et al., the frequency of intermediate alleles was only 1.9%. This difference was unexpected, given the similar definition of controls.

A priori, one would not expect a phenotype to be associated with FRAXA intermediate alleles. There is inconsistent evidence for a cognitive and/or behavioral phenotype related to premutation males and females (see Introduction). This is not surprising, given that premutation alleles are not hypermethylated and seem to produce amounts of FMRP similar to those produced by normal alleles (Feng et al. 1995). If a phenotype is not observed among premutation carriers, then one may not be expected among individuals with intermediate alleles. However, there is a strong correlation between premature menopause and female premutation carriers only (see Introduction). This association argues for the deleterious function of a large CGG repeat. Recent evidence regarding another trinucleotide-repeat disease, DM, suggests a mechanism for a gain of function resulting from an increased number of repeats. Philips et al. (1998) found that the normal activity of a CUG-binding protein was disrupted as the number of repeats in the RNA of the *DMPK* gene increased. This resulted in the abnormal splicing of a certain family of genes. Since there are known CGG-specific DNA-binding proteins (Deissler et al. 1996), this type of mechanism could induce altered expression related to increased repeat numbers at the FRAXA locus.

On the basis of our study, the FRAXA and FRAXE intermediate/premutation alleles do not seem to have an impact on IQ. Clearly, more studies using proper case and control populations, with large sample sizes, are needed, to resolve this contradictory observation.

**Table 8**  
**Test of Homogeneity: Frequency of Intermediate/Premutation Alleles among Cases and Controls, by District and by IQ**

| PARAMETER | ALLELE FREQUENCY   |              |                    |             |
|-----------|--------------------|--------------|--------------------|-------------|
|           | FRAXA, ≥41 Repeats |              | FRAXE, ≥31 Repeats |             |
|           | Cases              | Controls     | Cases              | Controls    |
| District: |                    |              |                    |             |
| 1         | 17/303 (.06)       | 6/303 (.02)  | 3/256 (.01)        | 1/256 (.01) |
| 2         | 4/164 (.02)        | 9/164 (.06)  | 0/136              | 1/136 (.01) |
| 3         | 3/97 (.03)         | 4/97 (.04)   | 1/65 (.01)         | 0/65        |
| 4         | 9/280 (.03)        | 10/280 (.04) | 0/199              | 2/199 (.01) |
| IQ:       |                    |              |                    |             |
| ≥70       | 29/652 (.04)       | 22/652 (.03) | 3/517 (.01)        | 3/517 (.01) |
| <70       | 3/102 (.03)        | 3/102 (.03)  | 1/68 (.01)         | 1/68 (.01)  |

**Acknowledgments**

We would like to acknowledge Lori Griffin for the establishment of lymphoblastoid cell lines. We thank Sheila Youings, Anna Murray, and Patricia Jacobs for both technical assistance and intellectual discussions involving our work, as well as for their unpublished data. We also thank Steve Warren for critical reading of this manuscript. Finally, we thank the participating parents and children, whose cooperation made this study possible. This work was supported by National Institutes of Health grant HD29909.

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